Translocation of a β-Peptide Across Cell Membranes

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Supporting Information

*Synthesis and purification of **1**.*

β-Peptides such as **1** can be conveniently prepared with conventional automated solid-phase synthesis equipment, although the coupling and deprotection steps require more time than do analogous steps in α-peptide synthesis. Preparation of **1** was challenging because 6 of the 11 residues are β^3-homoarginine (β^3hArg). The protected (*S*)-β^3hArg monomer was synthesized by Arndt-Eistert homologation,[14] as modified by Müller et al.,[15] starting from Fmoc-L-Arg with 2,2,5,7,8-pentamethyl-chroman-6-sulfonyl (Pmc) protection of the sidechain guanidino group. Conventional purification provided Fmoc-(*S*)-β^3hArg(Pmc) contaminated with small amounts (≤ 1%) of Fmoc-L-Arg(Pmc). It was impossible to detect this impurity by NMR or TLC; use of impure Fmoc-(*S*)-β^3hArg(Pmc) in syntheses of **1** led to α-Arg-containing oligomer contaminants that could be detected by mass spectrometry but were impossible to separate by HPLC. This problem was circumvented by developing an HPLC assay to monitor Fmoc-L-Arg(Pmc) impurity levels. Only Fmoc-(*S*)-β^3hArg(Pmc) without detectable impurity (< 0.05%), obtained via repeated column chromatography, was used for oligomer synthesis.

HPLC assay for Fmoc-β^3-homoarginine(Pmc) purity: Alltech C-18 analytical column (Waters Spherisorb ODS-2 5U), 9:1 solvent A:solvent B to 2:8 solvent A:solvent B over 70 min, followed by a 10 min flush with 5:95 solvent A:solvent B and a 10 min flush with 9:1 solvent A:solvent B, where solvent A = 100 mM aqueous NaOAc (pH 6.5) containing 10% (v/v) CH_3CN, and solvent B = CH_3CN containing 10% (v/v) water. Under these conditions Fmoc-β^3HArg(Pmc) and Fmoc-L-Arg(Pmc) elute between 40 and 50 min with retention times that differ by ca. 4 min.

Additional confocal microscopy image.

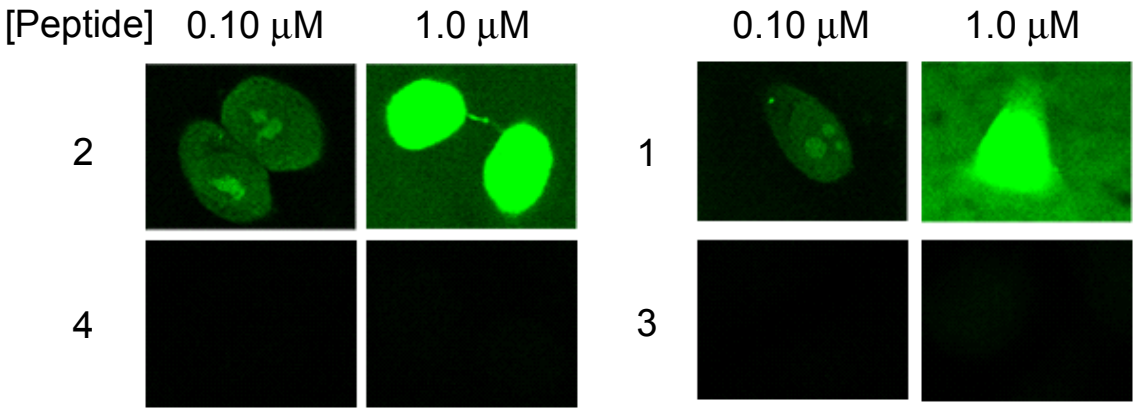

Figure S1. Confocal microscopy images of HeLa cells incubated for 10 min at 37 ºC with a solution 0.10 μM fluorescein-labeled peptides **1**, **2**, **3**, or **4**, washed, and fixed as for Figure 1 in the text. The dark panels show that peptides **3** and **4** are not internalized, even at 1.0 μM. The microscope settings were identical for each peptide and dose. Bar: 10 μm.

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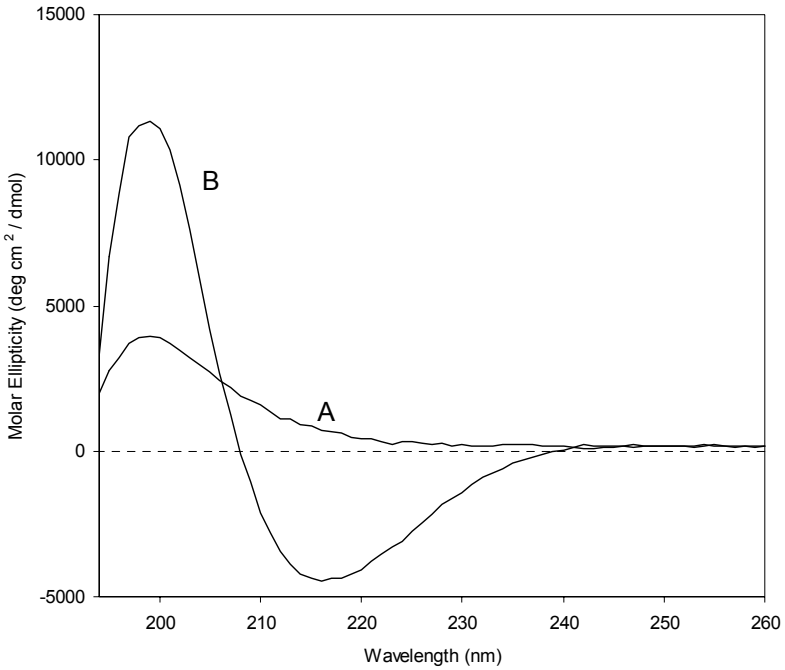

Figure S2. Circular dichroism data obtained on an Aviv 202SF instrument with 1-mm pathlength cells at 25 ºC for a solution of 0.12 mM β-peptide **5** in water (A) and methanol (B); data are shown as mean residue ellipticity. Several studies suggest that the random coil state of β-peptides displays no characteristic CD maxima or minima in the far-UV region,[A] but **5** in water displays a maximum at 199 nm. This CD spectrum is reminiscent of the signature for the 10/12-helix (defined by alternating 10-membered ring i → i-1 C=O--HN hydrogen bonds and 12-membered ring i → i+3 C=O--HN hydrogen bonds), which has a maximum at 205 nm.[B] The intensity of the CD maximum of **5** in aqueous solution, however, is quite low relative to 10/12-helix precedents in methanol,[B] which suggests that **5** is largely disordered in water. In organic solvents, many β-peptides constructed from β^3-residues adopt a 14-helical conformation (multiple 14-membered ring i → i-2 C=O--HN hydrogen bonds).[C] The stability of this conformation among β-peptides comprised of β^3-residues is much higher in methanol than in water.[A(i),D] In contrast to Tat 47-60, which remains unstructured in methanol,[6b] β-peptide **5** displays the characteristic 14-helical CD signature, with a minimum at 216 nm and a maximum at 199 nm. Thus, β-peptide **1** has an intrinsically higher propensity than does α-peptide **2** to adopt an ordered conformation in non-aqueous environments.

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[B] Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J. V. *Helv. Chim. Acta* 1998, *81*, 932. Seebach, D.; Gademann, K.; Schreiber, J. V.; Matthews, J. L.; Hintermann, T.; Jaun, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* 1997, *80*, 2033.

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